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(21) International Application Number: PCT/US99/00515 (22) International Filing Date: 8 January 1999 (08.01.99) (30) Priority Data: 60/071,073 9 January 1998 (09.01.98) US (71) Applicant (for all designated States except US): IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): SPRIGGS, Melanie [US/US]; 51 University Street, Seattle, WA 98101 (US). (74) Agents: FORDIS, Jean, B. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: IL-17RH DNA AND POLYPEPTIDES			
(57) Abstract DNA encoding IL-17RH polypeptides and methods for using the encoded polypeptides are disclosed.			

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IL-17RH DNA AND POLYPEPTIDES**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application Ser. No. 60/071,073, filed January 9, 1998, which is hereby incorporated by reference.

FIELD OF THE INVENTION

The invention is directed to purified and isolated IL-17RH polypeptides, the nucleic acids encoding such polypeptides, processes for production of recombinant forms of such polypeptides, antibodies generated against these polypeptides, fragmented peptides derived from these polypeptides, the use of such polypeptides and fragmented peptides as molecular weight markers, the use of such polypeptides and fragmented peptides as controls for peptide fragmentation, and kits comprising these reagents.

BACKGROUND OF THE INVENTION

The discovery and identification of proteins is at the forefront of modern molecular biology and biochemistry. The identification of the primary structure, or sequence, of a sample protein is the culmination of an arduous process of experimentation. In order to identify an unknown sample protein, the investigator can rely upon comparison of the unknown sample protein to known peptides using a variety of techniques known to those skilled in the art. For instance, proteins are routinely analyzed using techniques such as electrophoresis, sedimentation, chromatography, and mass spectrometry.

Comparison of an unknown protein sample to polypeptides of known molecular weight allows a determination of the apparent molecular weight of the unknown protein sample (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)). Protein molecular weight standards are commercially available to assist in the estimation of

molecular weights of unknown protein samples (New England Biolabs Inc. Catalog:130-131, 1995; J. L. Hartley, U.S. Patent No. 5,449,758). However, the molecular weight standards may not correspond closely enough in size to the unknown sample protein to allow an accurate estimation of apparent molecular weight.

5 The difficulty in estimation of molecular weight is compounded in the case of proteins that are subjected to fragmentation by chemical or enzymatic means (A.L. Lehninger, *Biochemistry* 106-108 (Worth Books, 2d ed. 1981)). Chemical fragmentation can be achieved by incubation of a protein with a chemical, such as cyanogen bromide, which leads to cleavage of the peptide bond on the carboxyl side of methionine residues (E. Gross, *Methods in Enz.* 11:238-10 255, 1967). Enzymatic fragmentation of a protein can be achieved by incubation of a protein with a protease that cleaves at multiple amino acid residues (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977). Enzymatic fragmentation of a protein can also be achieved by incubation of a protein with a protease, such as *Achromobacter* protease I (F. Sakiyama and A. Nakata, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. 15 Masaki et al., *Biochim. Biophys. Acta* 660:51-55, 1981), which leads to cleavage of the peptide bond on the carboxyl side of lysine residues. The molecular weights of the fragmented peptides can cover a large range of molecular weights and the peptides can be numerous. Variations in the degree of fragmentation can also be accomplished (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977).

20 The unique nature of the composition of a protein with regard to its specific amino acid constituents results in a unique positioning of cleavage sites within the protein. Specific fragmentation of a protein by chemical or enzymatic cleavage results in a unique "peptide fingerprint" (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977; M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980). Consequently, cleavage at specific sites results in reproducible

fragmentation of a given protein into peptides of precise molecular weights. Furthermore, these peptides possess unique charge characteristics that determine the isoelectric pH of the peptide. These unique characteristics can be exploited using a variety of electrophoretic and other techniques (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d
5 ed. 1991)).

When a peptide fingerprint of an unknown protein is obtained, this can be compared to a database of known proteins to assist in the identification of the unknown protein (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; B. Thiede et al., *Electrophoresis* 1996, 17:588-599, 1996). A variety of computer software programs are accessible via the Internet to
10 the skilled artisan for the facilitation of such comparisons, such as MultiIdent (Internet site: www.expasy.ch/sprot/multiident.html), PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearchForm.html), and ProFound (Internet site: www.chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag.html). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a
15 designated tolerance. The programs compare these molecular weights to protein databases to assist in the elucidation of the identity of the sample protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in the determination of the number of fragmented peptides and the precise molecular weight of those peptides should result
20 in enhanced success in the identification of unknown proteins.

Fragmentation of proteins is further employed for the production of fragments for amino acid composition analysis and protein sequencing (P. Matsudaira, *J. Biol. Chem.* 262:10035-10038, 1987; C. Eckerskorn et al., *Electrophoresis* 1988, 9:830-838, 1988), particularly the production of fragments from proteins with a "blocked" N-terminus. In addition, fragmentation

of proteins can be used in the preparation of peptides for mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; B. Thiede et al., *Electrophoresis* 1996, 17:588-599, 1996), for immunization, for affinity selection (R. A. Brown, U.S. Patent No. 5,151,412), for determination of modification sites (e.g. phosphorylation), for generation of active biological
5 compounds (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 300-301 (Prentice Hall, 6d ed. 1991)), and for differentiation of homologous proteins (M. Brown et al., *J. Gen. Virol.* 50:309-316, 1980).

In view of the continuing interest in protein research and the elucidation of protein structure and properties, there exists a need in the art for polypeptides suitable for use in peptide
10 fragmentation studies and in molecular weight measurements.

SUMMARY OF THE INVENTION

The invention aids in fulfilling this need in the art. The invention encompasses an isolated nucleic acid molecule comprising the DNA sequence of SEQ ID NO:1 and an isolated nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:2. The invention also
15 encompasses nucleic acid molecules complementary to these sequences. As such, the invention includes double-stranded nucleic acid molecules comprising the DNA sequence of SEQ ID NO:1 and isolated nucleic acid molecules encoding the amino acid sequence of SEQ ID NO:2. Both single-stranded and double-stranded RNA and DNA IL-17RH nucleic acid molecules are encompassed by the invention. These molecules can be used to detect both single-stranded and
20 double-stranded RNA and DNA variants of IL-17RH encompassed by the invention. A double-stranded DNA probe allows the detection of nucleic acid molecules equivalent to either strand of the nucleic acid molecule. Isolated nucleic acid molecules that hybridize to a denatured, double-stranded DNA comprising the DNA sequence of SEQ ID NO:1 or an isolated nucleic acid

molecule encoding the amino acid sequence of SEQ ID NO:2 under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS are encompassed by the invention.

The invention further encompasses isolated nucleic acid molecules derived by *in vitro* mutagenesis from SEQ ID NO:1. *In vitro* mutagenesis would include numerous techniques known in the art including, but not limited to, site-directed mutagenesis, random mutagenesis, and *in vitro* nucleic acid synthesis. The invention also encompasses isolated nucleic acid molecules degenerate from SEQ ID NO:1 as a result of the genetic code, isolated nucleic acid molecules which are allelic variants of human IL-17RH DNA or a species homolog of IL-17RH DNA. The invention also encompasses recombinant vectors that direct the expression of these nucleic acid molecules and host cells transformed or transfected with these vectors.

The invention also encompasses isolated polypeptides encoded by these nucleic acid molecules, including isolated polypeptides having a molecular weight of approximately 27 kD as determined by SDS-PAGE and isolated polypeptides in non-glycosylated form. Isolated polyclonal or monoclonal antibodies that bind to these polypeptides are encompassed by the invention. The invention further encompasses methods for the production of IL-17RH polypeptides including culturing a host cell under conditions promoting expression and recovering the polypeptide from the culture medium. Especially, the expression of IL-17RH polypeptides in bacteria, yeast, plant, and animal cells is encompassed by the invention.

In addition, assays utilizing IL-17RH polypeptides to screen for potential inhibitors of activity associated with IL-17RH polypeptide counter-structure molecules, and methods of using IL-17RH polypeptides as therapeutic agents for the treatment of diseases mediated by IL-17RH polypeptide counter-structure molecules are encompassed by the invention. Further, methods of

using IL-17RH polypeptides in the design of inhibitors thereof are also an aspect of the invention.

The invention further encompasses the fragmented peptides produced from IL-17RH polypeptides by chemical or enzymatic treatment. In addition, forms of IL-17RH polypeptide
5 molecular weight markers and fragmented peptides thereof, wherein at least one of the sites necessary for fragmentation by chemical or enzymatic means has been mutated, are an aspect of the invention.

The invention also encompasses a method for the visualization of IL-17RH polypeptide molecular weight markers and fragmented peptides thereof using electrophoresis. The invention
10 further includes a method for using IL-17RH polypeptide molecular weight markers and fragmented peptides thereof as molecular weight markers that allow the estimation of the molecular weight of a protein or a fragmented protein sample. The invention further encompasses methods for using IL-17RH polypeptides and fragmented peptides thereof as markers, which aid in the determination of the isoelectric point of a sample protein. The
15 invention also encompasses methods for using IL-17RH polypeptides and fragmented peptides thereof as controls for establishing the extent of fragmentation of a protein sample.

Further encompassed by this invention are kits to aid the determination of molecular weights of a sample protein utilizing IL-17RH polypeptide molecular weight markers, fragmented peptides thereof, and forms of IL-17RH polypeptide molecular weight markers,
20 wherein at least one of the sites necessary for fragmentation by chemical or enzymatic means has been mutated.

BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be more fully described with reference to the drawings in which:

Figure 1 is the nucleotide sequence of IL-17RH DNA, SEQ ID NO:1.

Figure 2 is the amino acid sequence of IL-17RH polypeptide, SEQ ID NO:2.

DETAILED DESCRIPTION OF THE INVENTION

A cDNA encoding human IL-17RH polypeptide has been isolated and is disclosed in
5 SEQ ID NO:1. This discovery of the cDNA encoding human IL-17RH polypeptide enables
construction of expression vectors comprising nucleic acid sequences encoding IL-17RH
polypeptides; host cells transfected or transformed with the expression vectors; biologically
active human IL-17RH polypeptide and IL-17RH molecular weight markers as isolated and
purified proteins; and antibodies immunoreactive with IL-17RH polypeptides. In addition,
10 understanding of the mechanism by which IL-17RH functions in human IL-17 receptor (IL-17R)
signaling enables the design of assays to detect inhibitors of IL-17R activity.

IL-17RH DNA was originally seen as three partial EST clones in the public databases.
The ESTs were re-sequenced and found to be divergent from one another. The consensus cDNA
sequence of the three ESTs is given in SEQ ID NO:1. This cDNA lacks additional 5' coding
15 sequences.

SEQ ID NO:1 encodes IL-17RH polypeptide (SEQ ID NO:2), which exhibits 24%
identity and 47% similarity to IL-17R by computer homology analysis. Remarkably, the
homology occurs within a portion of the IL-17R known to be within the cytoplasmic domain.

Since IL-17RH polypeptide encodes a receptor, which is related to the IL-17R; it can
20 bind cytokines that are similar to IL-17, as well as other cytokines. The observed homology in
the cytoplasmic domain indicates that IL-17RH polypeptide is capable of signaling. Therefore,
IL-17RH polypeptide can induce NFkB and regulate the production of heterologous cytokines.

In one embodiment of this invention, the expression of recombinant IL-17RH polypeptides can be accomplished utilizing fusion of sequences encoding IL-17RH polypeptides to sequences encoding another polypeptide to aid in the purification of IL-17RH polypeptides. An example of such a fusion is a fusion of sequences encoding an IL-17RH polypeptide to sequences encoding the product of the *malE* gene of the pMAL-c2 vector of New England Biolabs, Inc. Such a fusion allows for affinity purification of the fusion protein, as well as separation of the maltose binding protein portion of the fusion protein from the IL-17RH polypeptide after purification. It is understood of course that many different vectors and techniques can be used for the expression and purification of IL-17RH polypeptides and that this embodiment in no way limits the scope of the invention.

The insertion of DNA encoding the IL-17RH polypeptide into the pMAL-c2 vector can be accomplished in a variety of ways using known molecular biology techniques. The preferred construction of the insertion contains a termination codon adjoining the carboxyl terminal codon of the IL-17RH polypeptide. In addition, the preferred construction of the insertion results in the fusion of the amino terminus of the IL-17RH polypeptide directly to the carboxyl terminus of the Factor Xa cleavage site in the pMAL-c2 vector. A DNA fragment can be generated by PCR using IL-17RH DNA as the template DNA and two oligonucleotide primers. Use of the oligonucleotide primers generates a blunt-ended fragment of DNA that can be isolated by conventional means. This PCR product can be ligated together with pMAL-p2 (digested with the restriction endonuclease *Xmn* I) using conventional means. Positive clones can be identified by conventional means. Induction of expression and purification of the fusion protein can be performed as per the manufacturer's instructions. This construction facilitates a precise separation of the IL-17RH polypeptide from the fused maltose binding protein utilizing a simple protease treatment as per the manufacturer's instructions. In this manner, purified IL-17RH

polypeptide can be obtained. Furthermore, such a constructed vector can be easily modified using known molecular biology techniques to generate additional fusion proteins.

Another preferred embodiment of the invention is the use of IL-17RH polypeptides as molecular weight markers to estimate the apparent molecular weight of a sample protein by gel electrophoresis. An isolated and purified IL-17RH polypeptide molecular weight marker according to the invention has a molecular weight of approximately 27,094 Daltons, in the absence of glycosylation. The IL-17RH polypeptide, together with a sample protein, can be resolved by denaturing polyacrylamide gel electrophoresis by conventional means (U. K. Laemmli, *Nature* 227:680-685, 1970) in two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 6-20%. Proteins on the gel can be visualized using a conventional staining procedure. The IL-17RH polypeptide molecular weight marker can be used as a molecular weight marker in the estimation of the apparent molecular weight of the sample protein. The unique amino acid sequence of IL-17RH (SEQ ID NO:2) specifies a molecular weight of approximately 27,094 Daltons. Therefore, the IL-17RH polypeptide molecular weight marker serves particularly well as a molecular weight marker for the estimation of the apparent molecular weight of sample proteins that have apparent molecular weights close to 27,094 Daltons. The use of this polypeptide molecular weight marker allows an increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 27,094 Daltons. It is understood of course that many different techniques can be used for the determination of the molecular weight of a sample protein using IL-17RH polypeptides and that this embodiment in no way limits the scope of the invention.

Another preferred embodiment of the invention is the use of IL-17RH fragmented peptide molecular weight markers, generated by chemical fragmentation of IL-17RH polypeptide, as molecular weight markers to estimate the apparent molecular weight of a sample protein by gel

electrophoresis. Isolated and purified IL-17RH polypeptide can be treated with cyanogen bromide under conventional conditions that result in fragmentation of the IL-17RH polypeptide molecular weight marker by specific hydrolysis on the carboxyl side of the methionine residues within the IL-17RH polypeptide (E. Gross, *Methods in Enz.* 11:238-255, 1967). Due to the
5 unique amino acid sequence of the IL-17RH polypeptide, the fragmentation of IL-17RH polypeptide molecular weight markers with cyanogen bromide generates a unique set of IL-17RH fragmented peptide molecular weight markers. The distribution of methionine residues determines the number of amino acids in each peptide and the unique amino acid composition of each peptide determines its molecular weight.

10 The unique set of IL-17RH fragmented peptide molecular weight markers generated by treatment of IL-17RH polypeptide with cyanogen bromide comprises 3 fragmented peptides of at least 10 amino acids in size. The peptide encoded by amino acids 1-112 of SEQ ID NO:2 has a molecular weight of approximately 13,175 Daltons. The peptide encoded by amino acids 113-205 of SEQ ID NO:2 has a molecular weight of approximately 10,451 Daltons. The peptide
15 encoded by amino acids 206-238 of SEQ ID NO:2 has a molecular weight of approximately 3,503 Daltons. Therefore, cleavage of the IL-17RH polypeptide by chemical treatment with cyanogen bromide generates a unique set of IL-17RH fragmented peptide molecular weight markers. The unique and known amino acid sequence of these IL-17RH fragmented peptides allows the determination of the molecular weight of these fragmented peptide molecular weight
20 markers. In this particular case, IL-17RH fragmented peptide molecular weight markers have molecular weights of approximately 13,175; 10,451; and 3,503 Daltons.

The IL-17RH fragmented peptide molecular weight markers, together with a sample protein, can be resolved by denaturing polyacrylamide gel electrophoresis by conventional means in two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of

acrylamide between 10-20%. Proteins on the gel can be visualized using a conventional staining procedure. The IL-17RH fragmented peptide molecular weight markers can be used as molecular weight markers in the estimation of the apparent molecular weight of the sample protein. The unique amino acid sequence of IL-17RH specifies a molecular weight of approximately 13,175; 10,451; and 3,503 Daltons for the IL-17RH fragmented peptide molecular weight markers. Therefore, the IL-17RH fragmented peptide molecular weight markers serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of sample proteins that have apparent molecular weights close to 13,175; 10,451; or 3,503 Daltons. Consequently, the use of these fragmented peptide molecular weight markers allows an increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 13,175; 10,451; or 3,503 Daltons.

In a further embodiment, the sample protein and the IL-17RH polypeptide can be simultaneously, but separately, treated with cyanogen bromide under conventional conditions that result in fragmentation of the sample protein and the IL-17RH polypeptide by specific hydrolysis on the carboxyl side of the methionine residues within the sample protein and the IL-17RH polypeptide. As described above, the IL-17RH fragmented peptide molecular weight markers generated by cleavage of the IL-17RH polypeptide with cyanogen bromide have molecular weights of approximately 13,175; 10,451; and 3,503 Daltons.

The fragmented peptides from both the IL-17RH polypeptide and the sample protein can be resolved by denaturing polyacrylamide gel electrophoresis by conventional means in two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 10-20%. Fragmented peptides on the gel can be visualized using a conventional staining procedure. The IL-17RH fragmented peptide molecular weight markers can be used as molecular weight markers in the estimation of the apparent molecular weight of the fragmented

proteins derived from the sample protein. As discussed above, the IL-17RH fragmented peptide molecular weight markers serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of fragmented peptides that have apparent molecular weights close to 13,175; 10,451; or 3,503 Daltons. Consequently, the use of these IL-17RH fragmented peptide molecular weight markers allows an increased accuracy in the determination of apparent molecular weight of fragmented peptides that have apparent molecular weights close to 13,175; 10,451; or 3,503 Daltons. The extent of fragmentation of the IL-17RH polypeptide is further used as a control to determine the conditions expected for complete fragmentation of the sample protein. It is understood of course that many chemicals could be used to fragment IL-17RH polypeptides and that this embodiment in no way limits the scope of the invention.

In another embodiment, unique sets of IL-17RH fragmented peptide molecular weight markers can be generated from IL-17RH polypeptide using enzymes that cleave the polypeptide at specific amino acid residues. Due to the unique nature of the amino acid sequence of the IL-17RH polypeptide, cleavage at different amino acid residues will result in the generation of different sets of fragmented peptide molecular weight markers.

An isolated and purified IL-17RH polypeptide can be treated with *Achromobacter* protease I under conventional conditions that result in fragmentation of the IL-17RH polypeptide by specific hydrolysis on the carboxyl side of the lysine residues within the IL-17RH polypeptide (T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. Masaki et al., *Biochim. Biophys. Acta* 660:51-55, 1981). Due to the unique amino acid sequence of the IL-17RH polypeptide, the fragmentation of IL-17RH polypeptide molecular weight markers with *Achromobacter* protease I generates a unique set of IL-17RH fragmented peptide molecular weight markers. The distribution of lysine residues determines the number of amino acids in

each peptide and the unique amino acid composition of each peptide determines its molecular weight.

The unique set of IL-17RH fragmented peptide molecular weight markers generated by treatment of IL-17RH polypeptide with *Achromobacter* protease I comprises 12 fragmented peptides of at least 10 amino acids in size. The generation of 12 fragmented peptides with this enzyme treatment of the IL-17RH polypeptide, compared to 3 fragmented peptides with cyanogen bromide treatment of the IL-17RH polypeptide, clearly illustrates that both the size and number of the fragmented peptide molecular weight markers will vary depending upon the fragmentation treatment utilized to fragment the IL-17RH polypeptide. Both the size and number of these fragments are dictated by the amino acid sequence of the IL-17RH polypeptide.

The peptide encoded by amino acids 15-24 of SEQ ID NO:2 has a molecular weight of approximately 1,167 Daltons. The peptide encoded by amino acids 25-45 of SEQ ID NO:2 has a molecular weight of approximately 2,635 Daltons. The peptide encoded by amino acids 46-55 of SEQ ID NO:2 has a molecular weight of approximately 1,192 Daltons. The peptide encoded by amino acids 57-69 of SEQ ID NO:2 has a molecular weight of approximately 1,404 Daltons. The peptide encoded by amino acids 170-103 of SEQ ID NO:2 has a molecular weight of approximately 4,096 Daltons. The peptide encoded by amino acids 109-122 of SEQ ID NO:2 has a molecular weight of approximately 1,570 Daltons. The peptide encoded by amino acids 128-146 of SEQ ID NO:2 has a molecular weight of approximately 1,968 Daltons. The peptide encoded by amino acids 147-178 of SEQ ID NO:2 has a molecular weight of approximately 3,615 Daltons. The peptide encoded by amino acids 179-190 of SEQ ID NO:2 has a molecular weight of approximately 1,530 Daltons. The peptide encoded by amino acids 191-201 of SEQ ID NO:2 has a molecular weight of approximately 1,223 Daltons. The peptide encoded by amino acids 207-219 of SEQ ID NO:2 has a molecular weight of approximately 1,416 Daltons.

The peptide encoded by amino acids 227-238 of SEQ ID NO:2 has a molecular weight of approximately 1,278 Daltons.

Therefore, cleavage of the IL-17RH polypeptide by enzymatic treatment with *Achromobacter* protease I generates a unique set of IL-17RH fragmented peptide molecular weight markers. The unique and known amino acid sequence of these fragmented peptides allows the determination of the molecular weight of these IL-17RH fragmented peptide molecular weight markers. In this particular case, these IL-17RH fragmented peptide molecular weight markers have molecular weights of approximately 1,167; 2,635; 1,192; 1,404; 4,096; 1,570; 1,968; 3,615; 1,530; 1,223; 1,416; and 1,278 Daltons.

Once again, the IL-17RH fragmented peptide molecular weight markers, together with a sample protein, can be resolved by denaturing polyacrylamide gel electrophoresis by conventional means in two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 10-20%. Proteins on the gel can be visualized using a conventional staining procedure. The IL-17RH fragmented peptide molecular weight markers can be used as molecular weight markers in the estimation of the apparent molecular weight of the sample protein. The IL-17RH fragmented peptide molecular weight markers serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of proteins that have apparent molecular weights close to 1,167; 2,635; 1,192; 1,404; 4,096; 1,570; 1,968; 3,615; 1,530; 1,223; 1,416; or 1,278 Daltons. The use of these fragmented peptide molecular weight markers allows an increased accuracy in the determination of apparent molecular weight of proteins that have apparent molecular weights close to 1,167; 2,635; 1,192; 1,404; 4,096; 1,570; 1,968; 3,615; 1,530; 1,223; 1,416; or 1,278 Daltons.

In another embodiment, the sample protein and the IL-17RH polypeptide can be simultaneously, but separately, treated with *Achromobacter* protease I under conventional

conditions that result in fragmentation of the sample protein and the IL-17RH polypeptide by specific hydrolysis on the carboxyl side of the lysine residues within the sample protein and the IL-17RH polypeptide. The IL-17RH fragmented peptide molecular weight markers and the fragmented peptides derived from the sample protein are resolved by denaturing polyacrylamide gel electrophoresis by conventional means in two separate lanes of a gel containing sodium dodecyl sulfate and a concentration of acrylamide between 10-20%. Fragmented peptides on the gel can be visualized using a conventional staining procedure. The IL-17RH fragmented peptide molecular weight markers can be used as molecular weight markers in the estimation of the apparent molecular weight of the sample protein. The IL-17RH fragmented peptide molecular weight markers serve particularly well as molecular weight markers for the estimation of the apparent molecular weight of fragmented peptides that have apparent molecular weights close to 1,167; 2,635; 1,192; 1,404; 4,096; 1,570; 1,968; 3,615; 1,530; 1,223; 1,416; or 1,278 Daltons. The use of these IL-17RH fragmented peptide molecular weight markers allows an increased accuracy in the determination of apparent molecular weight of fragmented peptides that have apparent molecular weights close to 1,167; 2,635; 1,192; 1,404; 4,096; 1,570; 1,968; 3,615; 1,530; 1,223; 1,416; or 1,278 Daltons. The extent of fragmentation of the IL-17RH polypeptide is further used as a control to determine the conditions expected for complete fragmentation of the sample protein. It is understood of course that many enzymes could be used to fragment IL-17RH polypeptides and that this embodiment in no way limits the scope of the invention.

In another embodiment, monoclonal and polyclonal antibodies against IL-17RH polypeptides can be generated. Balb/c mice can be injected intraperitoneally on two occasions at 3 week intervals with 10 µg of isolated and purified IL-17RH polypeptide or peptides based on the amino acid sequence of IL-17RH polypeptides in the presence of RIBI adjuvant (RIBI Corp., Hamilton, Montana). Mouse sera are then assayed by conventional dot blot technique or

antibody capture (ABC) to determine which animal is best to fuse. Three weeks later, mice are given an intravenous boost of 3 µg of the IL-17RH polypeptide or peptides, suspended in sterile PBS. Three days later, mice are sacrificed and spleen cells fused with Ag8.653 myeloma cells (ATCC) following established protocols. Briefly, Ag8.653 cells are washed several times in

5 serum-free media and fused to mouse spleen cells at a ratio of three spleen cells to one myeloma cell. The fusing agent is 50% PEG: 10% DMSO (Sigma). Fusion is plated out into twenty 96-well flat bottom plates (Corning) containing HAT supplemented DMEM media and allowed to grow for eight days. Supernatants from resultant hybridomas are collected and added to a 96-well plate for 60 minutes that is first coated with goat anti-mouse Ig. Following washes, ¹²⁵I-IL-

10 17RH polypeptide or peptides are added to each well, incubated for 60 minutes at room temperature, and washed four times. Positive wells can be subsequently detected by autoradiography at -70°C using Kodak X-Omat S film. Positive clones can be grown in bulk culture and supernatants are subsequently purified over a Protein A column (Pharmacia). It is understood of course that many techniques could be used to generate antibodies against IL-17RH

15 polypeptides and fragmented peptides thereof and that this embodiment in no way limits the scope of the invention.

In another embodiment, antibodies generated against IL-17RH and fragmented peptides thereof can be used in combination with IL-17RH polypeptide or fragmented peptide molecular weight markers to enhance the accuracy in the use of these molecular weight markers to

20 determine the apparent molecular weight and isoelectric point of a sample protein. IL-17RH polypeptide or fragmented peptide molecular weight markers can be mixed with a molar excess of a sample protein and the mixture can be resolved by two dimensional electrophoresis by conventional means. Polypeptides can be transferred to a suitable protein binding membrane, such as nitrocellulose, by conventional means.

Polypeptides on the membrane can be visualized using two different methods that allow a discrimination between the sample protein and the molecular weight markers. IL-17RH polypeptide or fragmented peptide molecular weight markers can be visualized using antibodies generated against these markers and conventional immunoblotting techniques. This detection is performed under conventional conditions that do not result in the detection of the sample protein. It is understood that it may not be possible to generate antibodies against all IL-17RH polypeptide fragments, since small peptides may not contain immunogenic epitopes. It is further understood that not all antibodies will work in this assay; however, those antibodies which are able to bind IL-17RH polypeptides and fragments can be readily determined using conventional techniques.

The sample protein is visualized using a conventional staining procedure. The molar excess of sample protein to IL-17RH polypeptide or fragmented peptide molecular weight markers is such that the conventional staining procedure predominantly detects the sample protein. The level of IL-17RH polypeptide or fragmented peptide molecular weight markers is such as to allow little or no detection of these markers by the conventional staining method. The preferred molar excess of sample protein to IL-17RH polypeptide molecular weight markers is between 2 and 100,000 fold. More preferably, the preferred molar excess of sample protein to IL-17RH polypeptide molecular weight markers is between 10 and 10,000 fold and especially between 100 and 1,000 fold.

The IL-17RH polypeptide or fragmented peptide molecular weight markers can be used as molecular weight and isoelectric point markers in the estimation of the apparent molecular weight and isoelectric point of the sample protein. The IL-17RH polypeptide or fragmented peptide molecular weight markers serve particularly well as molecular weight and isoelectric point markers for the estimation of apparent molecular weights and isoelectric points of sample

proteins that have apparent molecular weights and isoelectric points close to that of the IL-17RH polypeptide or fragmented peptide molecular weight markers. The ability to simultaneously resolve the IL-17RH polypeptide or fragmented peptide molecular weight markers and the sample protein under identical conditions allows for increased accuracy in the determination of the apparent molecular weight and isoelectric point of the sample protein. This is of particular interest in techniques, such as two dimensional electrophoresis, where the nature of the procedure dictates that any markers should be resolved simultaneously with the sample protein.

In another embodiment, IL-17RH polypeptide or fragmented peptide molecular weight markers can be used as molecular weight and isoelectric point markers in the estimation of the apparent molecular weight and isoelectric point of fragmented peptides derived by treatment of a sample protein with a cleavage agent. It is understood of course that many techniques can be used for the determination of molecular weight and isoelectric point of a sample protein and fragmented peptides thereof using IL-17RH polypeptide molecular weight markers and peptide fragments thereof and that this embodiment in no way limits the scope of the invention.

IL-17RH polypeptide molecular weight markers encompassed by invention can have variable molecular weights, depending upon the host cell in which they are expressed. Glycosylation of IL-17RH polypeptide molecular weight markers and peptide fragments thereof in various cell types can result in variations of the molecular weight of these markers, depending upon the extent of modification. The size of IL-17RH polypeptide molecular weight markers can be most heterogeneous with fragments of IL-17RH polypeptide derived from the extracellular portion of the polypeptide. Consistent molecular weight markers can be obtained by using polypeptides derived entirely from the transmembrane and cytoplasmic regions, pretreating with N-glycanase to remove glycosylation, or expressing the polypeptides in bacterial hosts.

The interaction between IL-17RH and its counter-structure enables screening for small molecules that interfere with the IL-17RH/IL-17RH counter-structure association and inhibit activity of IL-17RH or its counter-structure. For example, the yeast two-hybrid system developed at SUNY (described in U.S. Patent No. 5,283,173 to Fields et al.) can be used to

5 screen for inhibitors of IL-17RH as follows. IL-17RH and its counter-structure, or portions thereof responsible for their interaction, can be fused to the Gal4 DNA binding domain and Gal 4 transcriptional activation domain, respectively, and introduced into a strain that depends on Gal4 activity for growth on plates lacking histidine. Compounds that prevent growth can be screened in order to identify IL-1 inhibitors. Alternatively, the screen can be modified so that IL-

10 17RH/IL-17RH counter-structure interaction inhibits growth, so that inhibition of the interaction allows growth to occur. Another, *in vitro*, approach to screening for IL-17RH inhibition would be to immobilize one of the components (either IL-17RH or its counter-structure) in wells of a microtiter plate, and to couple an easily detected indicator to the other component. An inhibitor of the interaction is identified by the absence of the detectable indicator from the well.

15 In addition, IL-17RH polypeptides according to the invention are useful for the structure-based design of an IL-17RH inhibitor. Such a design would comprise the steps of determining the three-dimensional structure of such the IL-17RH polypeptide, analyzing the three-dimensional structure for the likely binding sites of substrates, synthesizing a molecule that incorporates a predictive reactive site, and determining the inhibiting activity of the molecule.

20 Antibodies immunoreactive with IL-17RH polypeptides, and in particular, monoclonal antibodies against IL-17RH polypeptides, are now made available through the invention. Such antibodies can be useful for inhibiting IL-17RH polypeptide activity *in vivo* and for detecting the presence of IL-17RH polypeptides in a sample.

As used herein, the term "IL-17RH polypeptides" refers to a genus of polypeptides that further encompasses proteins having the amino acid sequence 1-238 of SEQ ID NO:2, as well as those proteins having a high degree of similarity (at least 90% homology) with such amino acid sequences and which proteins are biologically active. In addition, IL-17RH polypeptides refers
5 to the gene products of the nucleotides 1-714 of SEQ ID NO:1.

The isolated and purified IL-17RH polypeptide according to the invention has a molecular weight of approximately 27,094 Daltons. It is understood that the molecular weight of IL-17RH polypeptides can be varied by fusing additional peptide sequences to both the amino and carboxyl terminal ends of IL-17RH polypeptides. Fusions of additional peptide sequences at
10 the amino and carboxyl terminal ends of IL-17RH polypeptides can be used to enhance expression of IL-17RH polypeptides or aid in the purification of the protein.

It is understood that fusions of additional peptide sequences at the amino and carboxyl terminal ends of IL-17RH polypeptides will alter some, but usually not all, of the fragmented peptides of IL-17RH polypeptides generated by enzymatic or chemical treatment.

15 It is understood that mutations can be introduced into IL-17RH polypeptides using routine and known techniques of molecular biology. It is further understood that a mutation can be designed so as to eliminate a site of proteolytic cleavage by a specific enzyme or a site of cleavage by a specific chemically induced fragmentation procedure. It is also understood that the elimination of the site will alter the peptide fingerprint of IL-17RH polypeptides upon
20 fragmentation with the specific enzyme or chemical procedure.

The term "isolated and purified" as used herein, means that the IL-17RH polypeptide molecular weight markers or fragments thereof are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant host cell culture or as a purified product from a non-recombinant source. The term "substantially purified" as

used herein, refers to a mixture that contains IL-17RH polypeptide molecular weight markers or fragments thereof and is essentially free of association with other proteins or polypeptides, but for the presence of known proteins that can be removed using a specific antibody, and which substantially purified IL-17RH polypeptides or fragments thereof can be used as molecular weight markers. The term "purified" refers to either the "isolated and purified" form of IL-17RH polypeptides or the "substantially purified" form of IL-17RH polypeptides, as both are described herein.

A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct, that has been derived from DNA or RNA isolated at least once in substantially pure form (i.e., free of contaminating endogenous materials) and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

An IL-17RH polypeptide "variant" as referred to herein means a polypeptide substantially homologous to native IL-17RH polypeptides, but which has an amino acid sequence different from that of native IL-17RH polypeptides (human, murine or other mammalian species) because of one or more deletions, insertions or substitutions. The variant amino acid sequence preferably is at least 80% identical to a native IL-17RH polypeptide amino acid sequence, most preferably at least 90% identical. The percent identity can be determined, for example, by comparing

sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math* 5 2:482, 1981). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for 10 each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Variants can comprise conservatively substituted sequences, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, 15 Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Naturally occurring IL-17RH variants are also encompassed by the invention.

Examples of such variants are proteins that result from alternate mRNA splicing events or from 20 proteolytic cleavage of the IL-17RH polypeptides. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the IL-17RH polypeptides (generally from 1-5 terminal amino acids).

As stated above, the invention provides isolated and purified, or homogeneous, IL-17RH polypeptides, both recombinant and non-recombinant. Variants and derivatives of native IL-17RH polypeptides that can be used as molecular weight markers can be obtained by mutations of nucleotide sequences coding for native IL-17RH polypeptides. Alterations of the native amino acid sequence can be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985); Kunkel et al. (*Methods in Enzymol.* 154:367, 1987); and U.S. Patent Nos. 4,518,584 and 4,737,462, all of which are incorporated by reference.

IL-17RH polypeptides can be modified to create IL-17RH polypeptide derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, polyethylene glycol (PEG) groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of IL-17RH polypeptides can be prepared by linking the chemical moieties to functional groups on IL-17RH polypeptide amino acid side chains or at the N-terminus or C-terminus of an IL-17RH polypeptide or the extracellular domain thereof. Other derivatives of IL-17RH polypeptides within the scope of this invention include covalent or aggregative

conjugates of IL-17RH polypeptides or peptide fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugate can comprise a signal or leader polypeptide sequence (e.g. the α -factor leader of *Saccharomyces*) at the N-terminus of an IL-17RH polypeptide. The signal or leader peptide co-translationally or post-translationally directs transfer of the conjugate from its site of synthesis to a site inside or outside of the cell membrane or cell wall.

IL-17RH polypeptide conjugates can comprise peptides added to facilitate purification and identification of IL-17RH polypeptides. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988.

The invention further includes IL-17RH polypeptides with or without associated native-pattern glycosylation. IL-17RH polypeptides expressed in yeast or mammalian expression systems (e.g., COS-1 or COS-7 cells) can be similar to or significantly different from a native IL-17RH polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of IL-17RH polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated IL-17RH polypeptides can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

Equivalent DNA constructs that encode various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences are encompassed by the invention. For example, N-glycosylation sites in the IL-17RH polypeptide extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in

eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846, hereby incorporated by reference.

In another example, sequences encoding Cys residues that are not essential for biological activity can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon renaturation. Other equivalents are prepared by modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding, or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

The invention further encompasses isolated fragments and oligonucleotides derived from the nucleotide sequence of SEQ ID NO:1. The invention also encompasses polypeptides encoded by these fragments and oligonucleotides.

Nucleic acid sequences within the scope of the invention include isolated DNA and RNA sequences that hybridize to the native IL-17RH nucleotide sequences disclosed herein under conditions of moderate or severe stringency, and which encode IL-17RH polypeptides. As used

herein, conditions of moderate stringency, as known to those having ordinary skill in the art, and as defined by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of 50% formamide, 6X SSC at 42°C (or other similar hybridization solution, such as Stark's solution, in 50% formamide at 42°C), and washing conditions of about 60°C, 0.5X SSC, 0.1% SDS. Conditions of high stringency are defined as hybridization conditions as above, and with washing at 68°C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt concentration can be adjusted as necessary according to factors such as the length of the probe.

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:1 and still encode an IL-17RH polypeptide having the amino acid sequence of SEQ ID NO:2. Such variant DNA sequences can result from silent mutations (e.g., occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides equivalent isolated DNA sequences encoding IL-17RH polypeptides, selected from: (a) DNA derived from the coding region of a native mammalian IL-17RH gene; (b) cDNA comprising the nucleotide sequence 1-714 of SEQ ID NO:1; (c) DNA capable of hybridization to a DNA of (a) under conditions of moderate stringency and which encodes IL-17RH polypeptides; and (d) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b) or (c) and which encodes IL-17RH polypeptides. IL-17RH polypeptides encoded by such DNA equivalent sequences are encompassed by the invention.

DNA that is equivalent to the DNA sequence of SEQ ID NO:1 will hybridize under moderately stringent conditions to the double-stranded native DNA sequence that encode

polypeptides comprising amino acid sequences of 1-238 of SEQ ID NO:2. Examples of IL-17RH polypeptides encoded by such DNA, include, but are not limited to, IL-17RH polypeptide fragments and IL-17RH polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or conservative amino acid substitution(s), as described above. IL-17RH polypeptides encoded by DNA derived from other mammalian species, wherein the DNA will hybridize to the complement of the DNA of SEQ ID NO:1 are also encompassed.

IL-17RH polypeptide-binding proteins, such as the anti-IL-17RH polypeptide antibodies of the invention, can be bound to a solid phase such as a column chromatography matrix or a similar substrate suitable for identifying, separating or purifying cells that express IL-17RH polypeptides on their surface. Adherence of IL-17RH polypeptide-binding proteins to a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with IL-17RH polypeptide-binding proteins and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has IL-17RH polypeptide-binding proteins thereon. Cells having IL-17RH polypeptides on their surface bind to the fixed IL-17RH polypeptide-binding protein and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening or separating such IL-17RH polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner.

Alternatively, mixtures of cells suspected of containing IL-17RH polypeptide-expressing cells first can be incubated with a biotinylated IL-17RH polypeptide-binding protein. Incubation periods are typically at least one hour in duration to ensure sufficient binding to IL-17RH polypeptides. The resulting mixture then is passed through a column packed with avidin-coated

beads, whereby the high affinity of biotin for avidin provides the binding of the IL-17RH polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.*, 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

5 In the methods described above, suitable IL-17RH polypeptide-binding proteins are anti-IL-17RH polypeptide antibodies, and other proteins that are capable of high-affinity binding of IL-17RH polypeptides. A preferred IL-17RH polypeptide-binding protein is an anti-IL-17RH polypeptide monoclonal antibody.

IL-17RH polypeptides can exist as oligomers, such as covalently linked or non-
10 covalently linked dimers or trimers. Oligomers can be linked by disulfide bonds formed between cysteine residues on different IL-17RH polypeptides. In one embodiment of the invention, an IL-17RH polypeptide dimer is created by fusing IL-17RH polypeptides to the Fc region of an antibody (e.g., IgG1) in a manner that does not interfere with biological activity of IL-17RH polypeptides. The Fc polypeptide preferably is fused to the C-terminus of a soluble IL-17RH
15 polypeptide (comprising only the extracellular domain). General preparation of fusion proteins comprising heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991) and Byrn et al. (*Nature* 344:677, 1990), hereby incorporated by reference. A gene fusion encoding the IL-17RH polypeptide:Fc fusion protein is inserted into an appropriate expression
20 vector. IL-17RH polypeptide:Fc fusion proteins are allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between Fc polypeptides, yielding divalent IL-17RH polypeptides. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an IL-17RH polypeptide oligomer with as many as four IL-

17RH polypeptides extracellular regions. Alternatively, one can link two soluble IL-17RH polypeptide domains with a peptide linker.

Recombinant expression vectors containing a nucleic acid sequence encoding IL-17RH polypeptides can be prepared using well known methods. The expression vectors include an IL-
5 17RH DNA sequence operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are "operably linked" when the
10 regulatory sequence functionally relates to the IL-17RH DNA sequence. Thus, a promoter nucleotide sequence is operably linked to an IL-17RH DNA sequence if the promoter nucleotide sequence controls the transcription of the IL-17RH DNA sequence. The ability to replicate in the desired host cells, usually conferred by an origin of replication, and a selection gene by which transformants are identified can additionally be incorporated into the expression vector.

15 In addition, sequences encoding appropriate signal peptides that are not naturally associated with IL-17RH polypeptides can be incorporated into expression vectors. For example, a DNA sequence for a signal peptide (secretory leader) can be fused in-frame to the IL-17RH nucleotide sequence so that the IL-17RH polypeptide is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host
20 cells enhances extracellular secretion of the IL-17RH polypeptide. The signal peptide can be cleaved from the IL-17RH polypeptide upon secretion of IL-17RH polypeptide from the cell.

Suitable host cells for expression of IL-17RH polypeptides include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described, for example, in Pouwels et al.

Cloning Vectors: A Laboratory Manual, Elsevier, New York, (1985). Cell-free translation systems could also be employed to produce IL-17RH polypeptides using RNAs derived from DNA constructs disclosed herein.

Prokaryotes include gram negative or gram positive organisms, for example, *E. coli* or
5 *Bacilli*. Suitable prokaryotic host cells for transformation include, for example, *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various other species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In a prokaryotic host cell, such as *E. coli*, an IL-17RH polypeptide can include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met can be cleaved from
10 the expressed recombinant IL-17RH polypeptide.

Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those
15 derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. To construct an expression vector using pBR322, an appropriate promoter and an IL-17RH DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine
20 Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). Other commercially available vectors include those that are specifically designed for the expression of proteins; these would include pMAL-p2 and pMAL-c2 vectors that are used for the expression of proteins fused to maltose binding protein (New England Biolabs, Beverly, MA, USA).

Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β -lactamase (penicillinase), lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EP-A-36776), and tac promoter (Maniatis, 5 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful prokaryotic host cell expression system employs a phage λ P_L promoter and a cI857ts thermolabile repressor sequence. Plasmid vectors available from the American Type Culture Collection, which incorporate derivatives of the λ P_L promoter, include plasmid pHUB2 (resident in *E. coli* strain JMB9 (ATCC 37092)) and pPLc28 (resident in *E. coli* RR1 (ATCC 10 53082)).

IL-17RH DNA may be cloned in-frame into the multiple cloning site of an ordinary bacterial expression vector. Ideally the vector would contain an inducible promoter upstream of the cloning site, such that addition of an inducer leads to high-level production of the recombinant protein at a time of the investigator's choosing. For some proteins, expression 15 levels may be boosted by incorporation of codons encoding a fusion partner (such as hexahistidine) between the promoter and the gene of interest. The resulting "expression plasmid" may be propagated in a variety of strains of *E. coli*.

For expression of the recombinant protein, the bacterial cells are propagated in growth medium until reaching a pre-determined optical density. Expression of the recombinant protein 20 is then induced, e.g. by addition of IPTG (isopropyl-b-D-thiogalactopyranoside), which activates expression of proteins from plasmids containing a lac operator/promoter. After induction (typically for 1-4 hours), the cells are harvested by pelleting in a centrifuge, e.g. at 5,000 x G for 20 minutes at 4°C.

For recovery of the expressed protein, the pelleted cells may be resuspended in ten volumes of 50 mM Tris-HCl (pH 8)/1 M NaCl and then passed two or three times through a French press. Most highly-expressed recombinant proteins form insoluble aggregates known as inclusion bodies. Inclusion bodies can be purified away from the soluble proteins by pelleting in a centrifuge at 5,000 x G for 20 minutes, 4°C. The inclusion body pellet is washed with 50 mM Tris-HCl (pH 8)/1% Triton X-100 and then dissolved in 50 mM Tris-HCl (pH 8)/8 M urea/ 0.1 M DTT. Any material that cannot be dissolved is removed by centrifugation (10,000 x G for 20 minutes, 20°C). The protein of interest will, in most cases, be the most abundant protein in the resulting clarified supernatant. This protein may be "refolded" into the active conformation by dialysis against 50 mM Tris-HCl (pH 8)/5 mM CaCl₂/5 mM Zn(OAc)₂/1 mM GSSG/0.1 mM GSH. After refolding, purification can be carried out by a variety of chromatographic methods such as ion exchange or gel filtration. In some protocols, initial purification may be carried out before refolding. As an example, hexahistidine-tagged fusion proteins may be partially purified on immobilized Nickel.

While the preceding purification and refolding procedure assumes that the protein is best recovered from inclusion bodies, those skilled in the art of protein purification will appreciate that many recombinant proteins are best purified out of the soluble fraction of cell lysates. In these cases, refolding is often not required, and purification by standard chromatographic methods can be carried out directly.

IL-17RH polypeptides alternatively can be expressed in yeast host cells, preferably from the *Saccharomyces* genus (e.g., *S. cerevisiae*). Other genera of yeast, such as *Pichia*, *K. lactis*, or *Kluyveromyces*, can also be employed. Yeast vectors will often contain an origin of replication sequence from a 2 μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a

selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980), or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate
5 dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Hitzeman, EPA-73,657 or in Fleer et al., *Gene*, 107:285-195 (1991); and van den Berg et al., *Bio/Technology*, 8:135-139 (1990). Another
10 alternative is the glucose-repressible ADH2 promoter described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). Shuttle vectors replicable in both yeast and *E. coli* can be constructed by inserting DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) into the above-described yeast vectors.

15 The yeast α -factor leader sequence can be employed to direct secretion of an IL-17RH polypeptide. The α -factor leader sequence is often inserted between the promoter sequence and the structural gene sequence. See, e.g., Kurjan et al., *Cell* 30:933, 1982; Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984; U. S. Patent 4,546,082; and EP 324,274. Other leader sequences suitable for facilitating secretion of recombinant polypeptides from yeast hosts are known to
20 those of skill in the art. A leader sequence can be modified near its 3' end to contain one or more restriction sites. This will facilitate fusion of the leader sequence to the structural gene.

Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978. The Hinnen et al. protocol selects for Trp⁺ transformants in a selective medium, wherein the selective medium

consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 $\mu\text{g/ml}$ adenine, and 20 $\mu\text{g/ml}$ uracil.

Yeast host cells transformed by vectors containing ADH2 promoter sequence can be grown for inducing expression in a "rich" medium. An example of a rich medium is one consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 $\mu\text{g/ml}$ adenine and 80 $\mu\text{g/ml}$ uracil. Derepression of the ADH2 promoter occurs when glucose is exhausted from the medium.

Mammalian or insect host cell culture systems could also be employed to express recombinant IL-17RH polypeptides. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Established cell lines of mammalian origin also can be employed. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., *Cell* 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, and BHK (ATCC CRL 10) cell lines, and the CV-1/EBNA-1 cell line (ATCC CRL 10478) derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991).

Established methods for introducing DNA into mammalian cells have been described (Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine (Gibco/BRL) or Lipofectamine-Plus, can be used to transfect cells (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using resistance to cytotoxic drugs as a selection method. Kaufman et al., *Meth. in*

Enzymology 185:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable host strain for DHFR selection can be CHO strain DX-B11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B11, and only
5 cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

Transcriptional and translational control sequences for mammalian host cell expression
10 vectors can be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host
15 cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers et al., *Nature* 273:113, 1978; Kaufman, *Meth. in Enzymology*, 1990). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* I site located in the SV40 viral origin of replication site is included.

20 Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris et al., *Animal Cell Technology*, 1997, pp. 529-534) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras et al., *J. Biol. Chem.* 257:13475-13491, 1982). The internal ribosome entry site (IRES) sequences of

viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, *Current Opinion in Genetics and Development* 3:295-300, 1993; Ramesh et al., *Nucleic Acids Research* 24:2697-2700, 1996). Expression of a heterologous cDNA as part of a dicistronic mRNA followed by the gene for a selectable marker (eg. DHFR) has been shown to improve
5 transfectability of the host and expression of the heterologous cDNA (Kaufman, *Meth. in Enzymology*, 1990). Exemplary expression vectors that employ dicistronic mRNAs are pTR-DC/GFP described by Mosser et al., *Biotechniques* 22:150-161, 1997, and p2A5I described by Morris et al., *Animal Cell Technology*, 1997, pp. 529-534.

A useful high expression vector, pCAVNOT, has been described by Mosley et al., *Cell*
10 59:335-348, 1989. Other expression vectors for use in mammalian host cells can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984, has
15 been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in U.S. Patent Application Serial No. 07/701,415, filed May 16, 1991, incorporated by reference herein. The vectors can be derived from retroviruses. In place of the native signal sequence, a heterologous signal sequence can be added, such as the signal sequence for IL-7 described in United States Patent 4,965,195; the signal sequence for IL-2 receptor
20 described in Cosman et al., *Nature* 312:768 (1984); the IL-4 signal peptide described in EP 367,566; the type I IL-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type H IL-1 receptor signal peptide described in EP 460,846.

An isolated and purified IL-17RH polypeptide molecular weight marker according to the invention can be produced by recombinant expression systems as described above or purified

from naturally occurring cells. IL-17RH polypeptides can be substantially purified, as indicated by a single protein band upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

One process for producing IL-17RH polypeptides comprises culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes an IL-17RH polypeptide under conditions sufficient to promote expression of the IL-17RH polypeptide. IL-17RH polypeptide is then recovered from culture medium or cell extracts, depending upon the expression system employed. As is known to the skilled artisan, procedures for purifying a recombinant protein will vary according to such factors as the type of host cells employed and whether or not the recombinant protein is secreted into the culture medium. For example, when expression systems that secrete the recombinant protein are employed, the culture medium first can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium.

Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, (e.g., silica gel having pendant methyl or other aliphatic groups) can be employed to further purify IL-17RH polypeptides. Some or all of the foregoing purification steps, in various combinations, are well known and can be employed to provide an isolated and purified recombinant protein.

It is possible to utilize an affinity column comprising an IL-17RH polypeptide-binding protein, such as a monoclonal antibody generated against IL-17RH polypeptides, to affinity-purify expressed IL-17RH polypeptides. IL-17RH polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized.

Recombinant protein produced in bacterial culture is usually isolated by initial disruption of the host cells, centrifugation, extraction from cell pellets if an insoluble polypeptide, or from the supernatant fluid if a soluble polypeptide, followed by one or more concentration, salting-out, ion exchange, affinity purification or size exclusion chromatography steps. Finally, RP-HPLC can be employed for final purification steps. Microbial cells can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Transformed yeast host cells are preferably employed to express IL-17RH polypeptides as secreted polypeptides in order to simplify purification. Secreted recombinant polypeptide from a yeast host cell fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Urdal et al. describe two sequential, reversed-phase HPLC steps for purification of recombinant human IL-2 on a preparative HPLC column.

IL-17RH polypeptide molecular weight markers can be analyzed by methods including sedimentation, gel electrophoresis, chromatography, and mass spectrometry. IL-17RH polypeptides can serve as molecular weight markers using such analysis techniques to assist in the determination of the molecular weight of a sample protein. A molecular weight determination of the sample protein assists in the identification of the sample protein.

IL-17RH polypeptides can be subjected to fragmentation into peptides by chemical and enzymatic means. Chemical fragmentation includes the use of cyanogen bromide to cleave under neutral or acidic conditions such that specific cleavage occurs at methionine residues (E. Gross, *Methods in Enz.* 11:238-255, 1967). This can further include further steps, such as a
5 carboxymethylation step to convert cysteine residues to an unreactive species. Enzymatic fragmentation includes the use of a protease such as Asparaginylendopeptidase, Arginylendopeptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, or Endoproteinase Lys-C under conventional conditions to result in cleavage at specific amino acid residues. Asparaginylendopeptidase can cleave specifically on
10 the carboxyl side of the asparagine residues present within IL-17RH polypeptides. Arginylendopeptidase can cleave specifically on the carboxyl side of the arginine residues present within IL-17RH polypeptides. *Achromobacter* protease I can cleave specifically on the carboxyl side of the lysine residues present within IL-17RH polypeptides (Sakiyama and Nakat, U.S. Patent No. 5,248,599; T. Masaki et al., *Biochim. Biophys. Acta* 660:44-50, 1981; T. Masaki
15 et al., *Biochim. Biophys. Acta* 660:51-55, 1981). Trypsin can cleave specifically on the carboxyl side of the arginine and lysine residues present within IL-17RH polypeptides. *Staphylococcus aureus* V8 protease can cleave specifically on the carboxyl side of the aspartic and glutamic acid residues present within IL-17RH polypeptides (D. W. Cleveland, *J. Biol. Chem.* 3:1102-1106, 1977). Endoproteinase Asp-N can cleave specifically on the amino side of the asparagine
20 residues present within IL-17RH polypeptides. Endoproteinase Lys-C can cleave specifically on the carboxyl side of the lysine residues present within IL-17RH polypeptides. Other enzymatic and chemical treatments can likewise be used to specifically fragment IL-17RH polypeptides into a unique set of specific peptide molecular weight markers.

The resultant fragmented peptides can be analyzed by methods including sedimentation, electrophoresis, chromatography, and mass spectrometry. The fragmented peptides derived from IL-17RH polypeptides can serve as molecular weight markers using such analysis techniques to assist in the determination of the molecular weight of a sample protein. Such a molecular weight determination assists in the identification of the sample protein. IL-17RH fragmented peptide molecular weight markers are preferably between 10 and 237 amino acids in size. More preferably, IL-17RH fragmented peptide molecular weight markers are between 10 and 100 amino acids in size. Even more preferable are IL-17RH fragmented peptide molecular weight markers between 10 and 50 amino acids in size and especially between 10 and 35 amino acids in size. Most preferable are IL-17RH fragmented peptide molecular weight markers between 10 and 20 amino acids in size.

Furthermore, analysis of the progressive fragmentation of IL-17RH polypeptides into specific peptides (D. W. Cleveland et al., *J. Biol. Chem.* 252:1102-1106, 1977), such as by altering the time or temperature of the fragmentation reaction, can be used as a control for the extent of cleavage of a sample protein. For example, cleavage of the same amount of IL-17RH polypeptide and sample protein under identical conditions can allow for a direct comparison of the extent of fragmentation. Conditions that result in the complete fragmentation of IL-17RH polypeptide can also result in complete fragmentation of the sample protein.

In addition, IL-17RH polypeptides and fragmented peptides thereof possess unique charge characteristics and, therefore, can serve as specific markers to assist in the determination of the isoelectric point of a sample protein or fragmented peptide using techniques such as isoelectric focusing. The technique of isoelectric focusing can be further combined with other techniques such as gel electrophoresis to simultaneously separate a protein on the basis of molecular weight and charge. An example of such a combination is that of two-dimensional

electrophoresis (T.D. Brock and M.T. Madigan, *Biology of Microorganisms* 76-77 (Prentice Hall, 6d ed. 1991)). IL-17RH polypeptides and fragmented peptides thereof can be used in such analyses as markers to assist in the determination of both the isoelectric point and molecular weight of a sample protein or fragmented peptide.

- 5 Kits to aid in the determination of apparent molecular weight and isoelectric point of a sample protein can be assembled from IL-17RH polypeptides and peptide fragments thereof. Kits also serve to assess the degree of fragmentation of a sample protein. The constituents of such kits can be varied, but typically contain IL-17RH polypeptide and fragmented peptide molecular weight markers. Also, such kits can contain IL-17RH polypeptides wherein a site
10 necessary for fragmentation has been removed. Furthermore, the kits can contain reagents for the specific cleavage of IL-17RH and the sample protein by chemical or enzymatic cleavage. Kits can further contain antibodies directed against IL-17RH polypeptides or fragments thereof.

- Antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to a target IL-17RH mRNA sequence (forming a
15 duplex) or to the IL-17RH sequence in the double-stranded DNA helix (forming a triple helix) can be made according to the invention. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of IL-17RH cDNA (SEQ ID NO:1). Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to create an antisense or a sense oligonucleotide, based upon a
20 cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

 Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of complexes that block translation (RNA) or transcription (DNA) by one of several means, including enhanced degradation of the duplexes, premature termination of

transcription or translation, or by other means. The antisense oligonucleotides thus can be used to block expression of IL-17RH polypeptides. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation), but retain sequence specificity to be able to bind to target nucleotide sequences. Other examples of sense or antisense oligonucleotides include those oligonucleotides that are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes can be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides can be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO_4 -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. Antisense or sense oligonucleotides are preferably introduced into a cell containing the target nucleic acid sequence by insertion of the antisense or sense oligonucleotide into a suitable retroviral vector, then contacting the cell with the retrovirus vector containing the inserted sequence, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see PCT Application US 90/02656).

Sense or antisense oligonucleotides also can be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as

described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide can be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

Isolated and purified IL-17RH polypeptides or a fragment thereof can also be useful itself as a therapeutic agent in inhibiting IL-1 or TNF signaling. IL-17RH polypeptides can be introduced into the intracellular environment by well-known means, such as by encasing the protein in liposomes or coupling it to a monoclonal antibody targeted to a specific cell type.

IL-17RH DNA, IL-17RH polypeptides, and antibodies against IL-17RH polypeptides can be used as reagents in a variety of research protocols. A sample of such research protocols are given in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, (1989). For example, these reagents can serve as markers for cell specific or tissue specific expression of RNA or proteins. Similarly, these reagents can be used to investigate constitutive and transient expression of IL-17RH RNA or polypeptides. IL-17RH DNA can be used to determine the chromosomal location of IL-17RH DNA and to map genes in relation to this chromosomal location. IL-17RH DNA can also be used to examine genetic heterogeneity and heredity through the use of techniques such as genetic fingerprinting, as well as to identify risks associated with genetic disorders. IL-17RH DNA can be further used to identify additional genes related to IL-17RH DNA and to establish evolutionary trees based on

the comparison of sequences. IL-17RH DNA and polypeptides can be used to select for those genes or proteins that are homologous to IL-17RH DNA or polypeptides, through positive screening procedures such as Southern blotting and immunoblotting and through negative screening procedures such as subtraction.

5 IL-17RH polypeptides can also be used as a reagent to identify (a) any protein that IL-17RH polypeptide regulates, and (b) other proteins with which it might interact. IL-17RH polypeptides could be used by coupling recombinant protein to an affinity matrix, or by using them as a bait in the 2-hybrid system.

When used as a therapeutic agent, IL-17RH polypeptides can be formulated into
10 pharmaceutical compositions according to known methods. IL-17RH polypeptides can be combined in admixture, either as the sole active material or with other known active materials, with pharmaceutically suitable diluents (e.g., Tris-HCl, acetate, phosphate), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences,
15 16th ed. 1980, Mack Publishing Co. In addition, such compositions can contain IL-17RH polypeptides complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability,
20 rate of *in vivo* release, and rate of *in vivo* clearance of IL-17RH polypeptides.

Within an aspect of the invention, IL-17RH polypeptides, and peptides based on the amino acid sequence of IL-17RH, can be utilized to prepare antibodies that specifically bind to IL-17RH polypeptides. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof such as F(ab')₂, and Fab fragments, as well as any

recombinantly produced binding partners. Antibodies are defined to be specifically binding if they bind IL-17RH polypeptides with a K_a of greater than or equal to about 10^7 M^{-1} . Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

5 Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well-known in the art. In general, purified IL-17RH polypeptides, or a peptide based on the amino acid sequence of IL-17RH polypeptides that is appropriately conjugated, is administered to the host animal typically through parenteral injection. The immunogenicity of IL-17RH
10 polypeptides can be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to IL-17RH polypeptides. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent
15 immuno-electrophoresis (CIEP), radioimmunoassay, radio-immunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

Monoclonal antibodies can be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and
20 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980. Briefly, the host animals, such as mice are injected intraperitoneally at least once, and preferably at least twice at about 3 week intervals with isolated and purified IL-17RH polypeptides or conjugated IL-17RH polypeptides, optionally in the presence of adjuvant. Mouse sera are then assayed by conventional dot blot

technique or antibody capture (IL-17RH) to determine which animal is best to fuse.

Approximately two to three weeks later, the mice are given an intravenous boost of IL-17RH polypeptides or conjugated IL-17RH polypeptides. Mice are later sacrificed and spleen cells fused with commercially available myeloma cells, such as Ag8.653 (ATCC), following

5 established protocols. Briefly, the myeloma cells are washed several times in media and fused to mouse spleen cells at a ratio of about three spleen cells to one myeloma cell. The fusing agent can be any suitable agent used in the art, for example, polyethylene glycol (PEG). Fusion is plated out into plates containing media that allows for the selective growth of the fused cells. The fused cells can then be allowed to grow for approximately eight days. Supernatants from
10 resultant hybridomas are collected and added to a plate that is first coated with goat anti-mouse Ig. Following washes, a label, such as, ^{125}I -IL-17RH polypeptides is added to each well followed by incubation. Positive wells can be subsequently detected by autoradiography. Positive clones can be grown in bulk culture and supernatants are subsequently purified over a Protein A column (Pharmacia).

15 The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3:1-9 (1990), which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a
20 specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7:394 (1989).

Other types of "antibodies" can be produced using the information provided herein in conjunction with the state of knowledge in the art. For example, antibodies that have been

engineered to contain elements of human antibodies that are capable of specifically binding IL-17RH polypeptides are also encompassed by the invention.

Once isolated and purified, the antibodies against IL-17RH polypeptides can be used to detect the presence of IL-17RH polypeptides in a sample using established assay protocols.

- 5 Further, the antibodies of the invention can be used therapeutically to bind to IL-17RH polypeptides and inhibit its activity *in vivo*.

The purified IL-17RH polypeptides according to the invention will facilitate the discovery of inhibitors of IL-17RH polypeptides. The use of a purified IL-17RH polypeptide in the screening of potential inhibitors thereof is important and can eliminate or reduce the possibility of interfering reactions with contaminants.

In addition, IL-17RH polypeptides can be used for structure-based design of IL-17RH polypeptide-inhibitors. Such structure-based design is also known as "rational drug design." The IL-17RH polypeptides can be three-dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance or homology modeling, all of which are well-known methods. The use of IL-17RH polypeptide structural information in molecular modeling software systems to assist in inhibitor design and inhibitor-IL-17RH polypeptide interaction is also encompassed by the invention. Such computer-assisted modeling and drug design can utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. For example, most of the design of class-specific inhibitors of metalloproteases has focused on attempts to chelate or bind the catalytic zinc atom. Synthetic inhibitors are usually designed to contain a negatively-charged moiety to which is attached a series of other groups designed to fit the specificity pockets of the particular protease. A particular method of the invention comprises analyzing the three dimensional structure of IL-

17RH polypeptides for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

The specification is most thoroughly understood in light of the teachings of the references cited within the specification, which are hereby incorporated by reference. The embodiments
5 within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan recognizes many other embodiments are encompassed by the claimed invention.

What is claimed is:

1. An isolated IL-17RH nucleic acid molecule selected from the group consisting of:
 - (a) the DNA sequence of SEQ ID NO:1;
 - (b) an isolated nucleic acid molecule encoding an amino acid sequence comprising the sequence of SEQ ID NO:2;
 - (c) an isolated nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS;
 - (d) an isolated nucleic acid molecule derived by *in vitro* mutagenesis from SEQ ID NO:1;
 - (e) an isolated nucleic acid molecule degenerate from SEQ ID NO:1 as a result of the genetic code; and
 - (f) an isolated nucleic acid molecule selected from the group consisting of human IL-17RH DNA, an allelic variant of human IL-17RH DNA, mouse IL-17RH DNA, an allelic variant of mouse IL-17RH DNA, and a species homolog of IL-17RH DNA, .
2. A recombinant vector that directs the expression of a nucleic acid molecule of claim 1.
3. An isolated polypeptide encoded by a nucleic acid molecule of claim 1.
4. An isolated polypeptide according to claim 3 having a molecular weight of approximately 27 kD as determined by SDS-PAGE.
5. An isolated polypeptide according to claim 3 in non-glycosylated form.
6. Isolated antibodies that bind to a polypeptide of claim 3.
7. Isolated antibodies according to claim 6, wherein the antibodies are monoclonal antibodies.

8. A host cell transfected or transduced with the vector of claim 2.

9. A method for the production of IL-17RH polypeptide comprising culturing a host cell of claim 8 under conditions promoting expression, and recovering the polypeptide from the culture medium.

10. The method of claim 9, wherein the host cell is selected from the group consisting of bacterial cells, yeast cells, plant cells, and animal cells.

11. A method for the determination of the molecular weight of a sample protein comprising comparing molecular weight of a sample protein with the molecular weight of a polypeptide of claim 3;

wherein the comparison of molecular weights comprises application of the sample protein and polypeptide to an acrylamide gel, resolution of the sample protein and polypeptide using an electrical current, and application to the gel of a detection reagent, which stains the sample protein and polypeptide.

12. A kit for the determination of the molecular weights of peptide fragments of a sample protein comprising the following:

a vessel;

a polypeptide of claim 3;

at least one enzyme selected from the group consisting of Asparaginylendopeptidase, Arginylendopeptidase, *Achromobacter* protease I, Trypsin, *Staphylococcus aureus* V8 protease, Endoproteinase Asp-N, and Endoproteinase Lys-C;

a mutated polypeptide from said polypeptide by *in vitro* mutagenesis, wherein a site of enzymatic cleavage by the selected enzyme has been removed; and

fragmented peptides derived from said peptide by enzymatic cleavage with the selected enzyme;

wherein said polypeptide and said sample protein are contacted with the selected protease; and wherein the protein, polypeptides, and fragmented peptides are visualized by application of the protein, polypeptides, and fragmented peptides to an acrylamide gel, resolution

of the protein, polypeptides, and fragmented peptides using an electrical current, and application to the gel of a detection reagent, which stains the protein, polypeptides, and fragmented peptides.

1/2

IL-17RH
SEQ ID NO:1

AGTGCAGAGGTCAAGTGTCCCTGTTCATATACAGCACTAAAAGTACTGAG
GTTACAATCACACACCTCAAAGTATTTTTTTTTTTTTTTTGAGACAGGGTC
TCACTCTGTTGCCCAGGCTGTTACAGATAACAAATGCTTTTCTCCTCTC
ACAGAAAGGATCAAGAAGACTTCCTTTTCTACCACCACACTACTGCCCCC
CATTAAGGTTCTTGTGGTTTACCCATCTGAAATATGTTTCCATCACACAA
TTTGTTACTTCACTGAATTTCTTCAAAACCATTGCAGAAGTGAGGTCATC
CTTGAAAAGTGGCAGAAAAAGAAAATAGCAGAGATGGGTCCAGTGCAGTG
GCTTGCCACTCAAAAGAAGGCAGCAGACAAAGTCGTCTTCCTTCTTTCCA
ATGACGTCAACAGTGTGTGCGATGGTACCTGTGGCAAGAGCGAGGGCAGT
CCCAGTGAGAACTCTCAAGACCTCTTCCCCCTTGCCTTTAACCTTTTCTG
CAGTGATCTAAGAAGCCAGATTCTATCTGCACAAATACGTGGTGGTCTACT
TTAGAGAGATTGATACAAAAGACGATTACAATGCTCTCAGTGTCTGCCCC
AAGTACCACCTCATGAAGGATGCCACTGCTTTCTGTGCAGAACTTCTCCA
TGTCAGCAGCAGGTGTCAGCAGGAAAAAGATCACAAAGCCTGCCACGATG
GCTGCTGCTCCTTG

FIG.1

2/2

IL-17RH
SEQ ID NO:2

SAEVKCPCSYTALKVLRRLQSHTSKYFFFFLRQGLTLLPRLFTDNKCFSP
TERIKKTSFSTTTLLPPIKVLVVYPSEICFHHTICYFTEFLQNHCRSEVI
LEKWQKKKIAEMGPVQWLATQKKAADKVVFLLSNDVNSVCDGTCGKSEGS
PSENSQDLFPLAFNLFCSDLRSQIHLHKYVVVYFREIDTKDDYNALSVCP
KYHLMKDATAFCAELLHVKQQVSAGKRSQACHDGCCSL

FIG.2

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(21) International Application Number: PCT/US99/00515 (22) International Filing Date: 8 January 1999 (08.01.99) (30) Priority Data: 60/071,073 9 January 1998 (09.01.98) US (71) Applicant (for all designated States except US): IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): SPRIGGS, Melanie [US/US]; 51 University Street, Seattle, WA 98101 (US). (74) Agents: FORDIS, Jean, B. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 10 September 1999 (10.09.99)
(54) Title: IL-17RH DNA AND POLYPEPTIDES (57) Abstract DNA encoding IL-17RH polypeptides and methods for using the encoded polypeptides are disclosed.		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/00515

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C07K16/28 C12N1/21 C12N5/10
C12N1/19 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL/GENBANK DATABASES Accession no AA287951 Sequence reference HS1191353 12 April 1997 STRAUSBERG R: "National Cancer Institute, Cancer Genome Project (CGAP), Tumor Gene Index" XP002106191	1-10
Y	see the whole document ---	11
X	EMBL/GENBANK DATABASES Accession no R74129 Sequence reference HS129140 9 June 1995 HILLIER L ETAL: "The WashU-Merck EST Project" XP002106192	1-10
Y	see the whole document ---	11
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

17 June 1999

Date of mailing of the international search report

02/07/1999

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INTERNATIONAL SEARCH REPORT

Inter national Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>SCOPES R: "Protein Purification" 1987, SPRINGER VERLAG, NEW YORK XP002106193 see page 287, last paragraph - page 288, paragraph 1</p> <p>---</p>	11
A	<p>US 5 449 758 A (HARTLEY JAMES L) 12 September 1995 cited in the application</p> <p>---</p>	
A	<p>WO 96 29408 A (IMMUNEX CORP) 26 September 1996</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/00515

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5449758	A	12-09-1995	NONE
WO 9629408	A	26-09-1996	
		AU 696775	B 17-09-1998
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		CA 2215394	A 26-09-1996
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		US 5869286	A 09-02-1999